PATENT
Atty. Docket No.: 18396/1074

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Doorbar	Examiner:	Not Yet Assigned
Serial No.:	10/008,524	Group Art Unit:	1645
Filed:	November 5, 2001		
Titled:	Improvements in or Relating to Screening for Papilloma Viruses	Conf. No.:	2747

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8a

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Kathleen Williams
Name of Person Faxing

Signature of Person Mailing Paper

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Commissioner for Patents
Washington, D.C. 20231

AMENDMENT

Sir:

This paper is submitted in response to the Notice to File Missing Parts mailed January 11, 2002 by the United States Patent and Trademark Office, in the above-referenced Patent Application.

In the Specification:

Please enter the following amendments:

1. On a new page, following the Claims, insert the following new section:

ABSTRACT

The invention relates to a method of screening for precursor lesions which can lead to cervical malignancy, methods of detecting and typing human papilloma virus infections, and reagents of use in these methods.

2. On page 4 of the Specification, please replace paragraph 3, lines 15-18 with the following:

--In a further aspect the invention provides an antibody molecule or an antigen-binding variant thereof, which binds specifically to HPV E4 protein in the region of amino acid residues RPIPKPSPWAPKKHRRLSDQDSQTP (SEQ ID NO: 4) of HPV16 E4 protein, or the corresponding hydrophilic acid/base-rich region of other HPV E4 proteins.--

3. On page 4 &5 of the Specification, please replace the section entitled "Brief Description of the Drawings" with the following:

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SEQ ID NO:34; PSPWAPKK, SEQ ID NO:35; SPWAPKKH, SEQ ID NO:36; PWAPKKHR, SEQ ID NO:37; WAPKKHRR, SEQ ID NO:38; APKKHRRL, SEQ ID NO:39;PKKHRRLS, SEQ ID NO:40; KKHRRLSS, SEQ ID NO:41; KHRRLSSD, SEQ ID NO:42; HRRLSSDQ, SEQ ID NO:43; RRLSSDQD, SEQ ID NO:44; RLSSDQDQ, SEQ ID NO:45; LSSDQDQS, SEQ ID NO:46; SSDQDQSQ, SEQ ID NO:47; SDQDQSQT, SEQ ID NO:48; DQDQSQTP, SEQ ID NO: 49; QDQSQTPE, SEQ ID NO:50; DQSQTPET, SEQ ID NO:51; QSQTPETP, SEQ ID NO:52; SQTPETPA, SEQ ID NO:53; QTPETPAT, SEQ ID NO:54; TPETPATP, SEQ ID NO:55; PETPATPL, SEQ ID NO:56; ETPATPLS, SEQ ID NO:57; TPATPLSC, SEQ ID NO:58; PATPLSCC, SEQ ID NO:59; ATPLSCCT, SEQ ID NO:60; TPLSCCTE, SEQ ID NO:61; PLSCCTET, SEQ ID NO:62; LSCCTETQ, SEQ ID NO:63; SCCTETQW, SEQ ID NO:64; CCTETQWT, SEQ ID NO:65; CTETQWTV; SEQ ID NO:66; TETQWTVL, SEQ ID NO 67; ETQWTVLQ, SEQ ID NO:68; TQWTVLQS, SEQ ID NO:69; QWTVLQSS, SEQ ID NO:70; WTVLQSSL, SEQ ID NO:71; TVLQSSLH, SEQ ID NO:72; VLQSSLHL, SEQ ID NO:73; LQSSLHLT, SEQ ID NO:74; QSSLHLTA, SEQ ID NO:75; SSLHLTAH, SEQ ID NO:76; SLHLTAHT; SEQ ID NO: 77; LHLTAHTK, SEQ ID NO:78; HLTAHTKD, SEQ ID NO: 79; LTAHTKDG, SEQ ID NO:80; TAHTKDGL, SEQ ID NO:81; AHTKDGLT, SEQ ID NO:82, HTKDGLTV, SEQ ID NO:83; TKDGLTVI, SEQ ID NO:84; KDGLTVIV, SEQ ID NO:85; DGLTVIVT, SEQ ID NO:86; GLTVIVTL, SEQ ID NO:87; LTVIVTLH, SEQ ID NO:88; and TVIVTLHP, SEQ ID NO:89.

Figure 1B shows the sequence of the E4 protein from HPV16 (top row, SEQ ID NO:90), HPV1 (bottom row, SEQ ID NO:91) and a consensus sequence (middle row, SEQ ID NO:92), and the binding sites of various antibodies or antigen-binding variants of antibodies;

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4. On page 8 and 9 of the Specification, please replace paragraphs 2,3, and 4, extending from lines 9-31 on page 8 and 1-3 on page 9 with the following:

-- The present invention moreover provides a particular region of the E4 protein to which 10 molecules (particularly antibody molecules or variants thereof) may bind with considerable specificity. Although homologous regions exist in all HPV E4 proteins. the region varies in amino acid sequence between HPVs of different types. The region corresponds to a peak of hydrophilicity in the E4 protein and is probably surface-exposed. The region is highly charged (acid/base-rich). In HPV type 16, the amino acid sequence of the region is (from N-terminal to C-terminal) RPIPKPSPWAPKKHRRLSDQDSQTP (SEQ ID NO: 4). Clearly the amino acid sequence of the E4 proteins of other HPV types will not necessarily be identical to that in type 16, but with the benefit of the present disclosure (e.g. figure 9) the corresponding region can readily be identified in other E4 proteins by those skilled in the art by use of conventional alignment and sequence comparison computer programs (about 65 of the 70 or so known HPV genomes have been cloned and sequenced).

Thus, in a third aspect the invention provides an antibody molecule, or an antigen binding variant thereof, which binds specifically to HPV E4 protein in the region of amino acid residues RPIPKPSPWAPKKHRRLSDQDSQTP (SEQ ID NO: 4) of HPV16 E4 protein, or the corresponding hydrophilic, acid/base-rich region of other HPV E4 proteins, preferably other than the antibody TVG 402 identified by Doorbar *et al,* (1992 Virology 187, 353-359).

Moreover, the invention provides the use of an antibody molecule, or an antigen binding variant thereof, which binds specifically to HPV E4 protein in the region of amino acid residues RPIPKPSPWAPKKHRRLSDQDSQTP (SEQ ID NO: 4) of HPV16 E4 protein, or the corresponding hydrophilic, acid/base-rich region of other HPV E4 proteins for the detection of HPV infections as described herein.--

5. On page 9 and 10 of the Specification, please replace paragraphs 2,3, and 4, extending from lines 16-31 on page 9 and 1-6 on page 10 with the following:

-- Preferably the antibody of the invention has a binding site, as identified by the SPOTS epitope mapping system within the region. RPIPKPSPWAPKKHR (SEQ ID NO: 167) (or the corresponding amino acid sequence from other HPV types). A particularly preferred molecule is

the Fab fragment TVG405, described further below, which binds to the epitope PKPSPWAPKKH(R) (SEQ ID NO: 168) with extremely high affinity and is of particular usefulness in the methods of the invention defined above.

The arginine residue indicated in brackets at the C-terminal of the TVG405 epitope is not essential for high affinity binding.

The Fab fragment TVG405 was isolated by the present inventor using phage display technology, as described below. Those skilled in the art will understand that different antibodies or Fab fragments may readily be obtained by using similar phage display techniques (and screening with E4 proteins or portions thereof), or by using more conventional immunisation techniques (e.g. immunising mice, rabbits, rats or the like with E4 protein or peptides corresponding to portions of the E4 protein) to obtain polyclonal antisera or monoclonal antibodies (using well known hybridoma techniques of Milstein *et al*). Complete antibody molecules can readily be prepared from Fab -encoding sequences (e.g. isolated by phage display techniques) using standard DNA manipulation techniques described by Sambrook *et al,* (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, NY, USA) to join appropriate DNA sequences.--

6. On page 15 of the Specification, please replace paragraph 3, extending from lines 15-19 on with the following:

-- Antibodies to the N-terminus of the protein are raised against the synthetic peptide MADPAAATKYPLC (SEQ ID NO: 169) after conjugation to thyroglobulin or keyhole limpet haemocyanin through its C-terminal cysteine residue. Conjugation is carried out using m-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as described by Green *et al* (1982).--

7. On page 17 and 18 of the Specification, please replace paragraph 2, extending from lines 26-30 on page 17 and 1-2 on page 18 with the following:

--It is found that the amino acid sequence of the CDR3 loops of the TVG 405 and TVG 407 Fabs are as follows:

TVG 405 heavy chain CDR3 sequence: LLRGAFDY (SEQ ID NO: 170)

light chain CDR3 sequence: NSRDSSGGNAV (SEQ ID NO: 171)

TVG 4Q7 heavy chain CDR3 sequence: LVQGSFDY (SEQ ID NO: 172)

light chain CDR3 sequence: QADSSTHV (SEQ ID NO: 173)--

8. On page 20 of the Specification, please replace paragraphs 2, 3 and 4, extending from lines 9-16 with the following:

-- The first peptide is ferritin. (SEQ ID NO: 1)

The second peptide is a keratin filament binding protein, which has the sequence set forth in (SEQ ID NO: 2).

The third polypeptide is a novel polypeptide recognised as a member of the DEAD box family of proteins, which contain the characteristic sequence motif DEAD (SEQ ID NO: 129). The sequence of the third polypeptide is shown in (SEQ ID NO: 3).--

9. On page 21 and 22 of the Specification, please replace paragraphs 1 and 2, extending from lines 9-31 on page 21 and line1 on page 22 with the following:

-- DNA oligonucleotides 73 bases in length, having a randomised portion of 26 bases, are used for the development of an aptamer capable of binding E4. A library of synthetic RNA oligonucleotides having the following structure is prepared:

5' CCTGTTGTGAGCCTCCTGTCGAA (26N) TTGAGCGTTTATTCTTGTCTCCC 3' (SEQ ID NO: 174)

Where N stands for any possible base in the random region. The random region is generated by using a mixture of all four nucleotides (ratio 6:5:5:4, A:C:G:T, to allow for differences in coupling efficiency) during the synthesis of each nucleotide in that stretch of the oligonucleotide library. The resulting complexity is theoretically 4^{26} molecules. The scale of synthesis (0.1μmol) followed by gel purification yields 8.8nmol which puts an absolute upper limit of approximately $5x10^{15}$ on the number of different molecules actually present.

PCR Amplification with a 5' primer that introduces the recognition site for T7 RNA Polymerase (5' TAATACGACTCACTATAGGGAGACAAGAATAAACGCTCAA 3') (SEQ ID NO: 175) and 3'primer (5' GCCTGTTGTGAGCCTCCTGTCGAA 3') (SEQ ID NO: 176) results in the following template for transcription:

5' TAATACGAACTCACTATAGGGAGACAAGAATAAACGCTCAA (26N) TTCGACAGGAGGCTCACAACAGGC 3' (SEQ ID NO: 177)

The RNA transcript itself has the following sequence:

5' GGGAGACAAGAAUAAACGCUCAA (26N) UUCGACAGGAGGCUCACAACAGGC 3' (SEQ ID NO: 178)--

In the Claims

Please amend the following claims:

9. A method according to claim 8, wherein the hydrophilic region is the region is the region which possesses the sequence RPIPKPSPWAPKKHRRLSSDQDSQTP (SEQ ID NO: 4) in HPV 16, or its homologue in other papilloma viruses.

10. A method according to claim 9, wherein the hydrophilic region is the region which possesses the sequence RPIPKPSPWAPKKHR (SEQ ID NO: 167) in HPV 16, or its homologue in other papilloma viruses.

11. A method according to claim 10, wherein the hydrophilic region is the region which possesses the sequence PKPSPWAPKKH(R) (SEQ ID NO: 168) in HPV 16, or its homologue in other papilloma viruses.

13. An antibody molecule, or an antigen-binding variant thereof, which binds specifically to HPV E4 protein in the region of amino acid residues RPIPKPSPWAPKKHRRLSSDQDSQTP (SEQ ID NO: 4) of HPV 16 E4 protein, or the corresponding hydrophilic, acid/base-rich region of other HPV E4 proteins.

Remarks

Applicant respectfully request entry of this amendment and further states that the amendment adds no new matter.

6/11/02
Date

Kathleen M. Williams
Reg. No. 34, 380
Customer No.: 29933
Attorney for Applicant
Palmer & Dodge LLP
111 Huntington Ave.
Boston,MA02199

Marked-up Version of Amendments:

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The second peptide is a keratin filament binding protein, which has the sequence set forth in [SEQ. ID. No. 2.] (SEQ ID NO: 2).

The third polypeptide is a novel polypeptide recognised as a member of the DEAD box family of proteins, which contain the characteristic sequence motif DEAD (SEQ ID NO: 129). The sequence of the third polypeptide is shown in [SEQ. ID. No. 3]. (SEQ ID NO: 3).--

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-- DNA oligonucleotides 73 bases in length, having a randomised portion of 26 bases, are used for the development of an aptamer capable of binding E4. A library of synthetic RNA oligonucleotides having the following structure is prepared:

5' CCTGTTGTGAGCCTCCTGTCGAA (26N) TTGAGCGTTTATTCTTGTCTCCC 3' (SEQ ID NO: 174)

Where N stands for any possible base in the random region. The random region is generated by using a mixture of all four nucleotides (ratio 6:5:5:4, A:C:G:T, to allow for differences in coupling efficiency) during the synthesis of each nucleotide in that stretch of the oligonucleotide library. The resulting complexity is theoretically 4^{26} molecules. The scale of synthesis (0.1μmol) followed by gel purification yields 8.8nmol which puts an absolute upper limit of approximately $5x10^{15}$ on the number of different molecules actually present.

PCR Amplification with a 5' primer that introduces the recognition site for T7 RNA Polymerase (5' TAATACGACTCACTATAGGGAGACAAGAATAAACGCTCAA 3') (SEQ ID NO: 175) and 3'primer (5' GCCTGTTGTGAGCCTCCTGTCGAA 3') (SEQ ID NO: 176) results in the following template for transcription:

5' TAATACGAACTCACTATAGGGAGACAAGAATAAACGCTCAA (26N) TTCGACAGGAGGCTCACAACAGGC 3' (SEQ ID NO: 177)

The RNA transcript itself has the following sequence:

5' GGGAGACAAGAAUAAACGCUCAA (26N) UUCGACAGGAGGCUCACAACAGGC 3' (SEQ ID NO: 178)--

In the Claims

Please amend the following claims:

9. A method according to claim 8, wherein the hydrophilic region is the region is the region which possesses the sequence RPIPKPSPWAPKKHRRLSSDQDSQTP (SEQ ID NO: 4) in HPV 16, or its homologue in other papilloma viruses.

10. A method according to claim 9, wherein the hydrophilic region is the region which possesses the sequence RPIPKPSPWAPKKHR (SEQ ID NO: 167) in HPV 16, or its homologue in other papilloma viruses.

11. A method according to claim 10, wherein the hydrophilic region is the region which possesses the sequence PKPSPWAPKKH(R) (SEQ ID NO: 168) in HPV 16, or its homologue in other papilloma viruses.

13. An antibody molecule, or an antigen-binding variant thereof, which binds specifically to HPV E4 protein in the region of amino acid residues RPIPKPSPWAPKKHRRLSSDQDSQTP (SEQ ID NO: 4) of HPV 16 E4 protein, or the corresponding hydrophilic, acid/base-rich region of other HPV E4 proteins.